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Benzene Increases Aneuploidy in the Lymphocytes of Exposed Workers: A Comparison of Data Obtained by Fluorescence in Situ Hybridization in Interphase and Metaphase Cells

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Benzene is an established human leukemogen that increases the level of chromosome aberrations in lymphocytes of exposed workers. Numerical aberrations (aneusomy) can be observed by fluorescence in situ hybridization (FISH) in both interphase and metaphase cells. Whereas interphase FISH allows nondividing cells to be analyzed, one advantage of metaphase FISH is that it can also detect structural changes. The present study compares the abilities of metaphase and interphase FISH to detect aneusomy of chromosomes 7 and 8 in healthy benzene-exposed human subjects. Metaphase and interphase cells from the peripheral blood of 43 workers exposed to benzene (median = 31 ppm, 8-hr TWA) and 44 frequency-matched controls were analyzed by FISH. Normal diploid cells contained two hybridization signals, whereas those that were potentially monosomic contained one, trisomic 3 and tetrasomic 4. The frequency of cells with one hybridization signal for chromosome 7 in metaphase spreads rose from 2.72 ± 0.19 (%, mean ± SE) in controls to 3.79 ± 0.63 in workers exposed to 31 or fewer ppm benzene and 5.9 ± 0.85 in those exposed to more than 31 ppm ($P_{trend} < 0.0001$). No similar dose-dependent increase in the frequency of cells with one hybridization signal was observed by interphase FISH, probably because of probe overlap artifact. Although significant dose-dependent increases in the frequency of cells with three hybridization signals for chromosome 7 were detected by both methods in the higher-exposed group, a larger, more significant difference was detected by metaphase FISH between controls and workers exposed to 31 or fewer ppm. Similar data were obtained for chromosome 8. Interphase and metaphase FISH were moderately correlated for three hybridization signals but not for one hybridization signal in chromosome 7 or 8. In general, metaphase FISH was more sensitive in detecting both monosomy and trisomy in the lymphocytes of exposed workers. Environ. Mol. Mutagen. 34:260–268, 1999.

Key words: benzene; biomarkers; chromosomal aberrations; epidemiology

INTRODUCTION

Benzene, an important industrial chemical and ubiquitous environmental pollutant, causes leukemia and other bone marrow disorders in humans [IARC, 1987]. Occupational exposure to benzene has been associated with increased levels of chromosome aberrations in human lymphocytes [Dean, 1985; Major et al., 1994]. Aneusomies of C-group chromosomes (chromosomes 6–12, X) have been observed in pancytopenic benzene-poisoned patients and benzene-induced cases of myelodysplastic syndromes and myelogenous leukemia [Aksoy, 1988; Wolman, 1977; Forni et al., 1971; Van den Berghe et al., 1979; Ding et al., 1983]. These numerical changes in C-group chromosomes, for example, monosomy 7 (the loss of a chromosome) and trisomy 8 (the gain of a chromosome) are also commonly detected in leukemia patients [Le Beau, 1990; Sandberg, 1990].

Traditionally, chromosome aberrations have been de-

Abbreviations: FISH, fluorescence in situ hybridization; TWA, time-weighted average; SD, standard deviation; SE, standard error; NS, not significant.

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tected through conventional cytogenetics. However, this procedure has several drawbacks for the detection of chromosome-specific aneusomy. For example, the cells must be cultured to make metaphase spreads, a limited number (25–100) of scorable cells can be examined, and recognition of specific chromosomes is problematic. These problems can now be overcome by using fluorescence in situ hybridization (FISH) to measure aneuploidy of specific chromosomes in large numbers of interphase cells and metaphase spreads [Eastmond and Pinkel, 1990; Gray and Pinkel, 1992].

FISH has several advantages over conventional cytogenetics, including selectivity of specific DNA probes, multiple color labeling, sensitivity of detection, and speed of microscopic analysis. Interphase FISH, in particular, offers several advantages over classical cytogenetics [Eastmond et al., 1995]. First, interphase FISH allows analysis of nondividing cells. Second, a much larger number of cells, potentially 1000 or more, may be analyzed. Third, the detection of aneuploidy is facilitated simply by counting the number of labeled regions representing a particular chromosome of interest within the isolated interphase nucleus. Metaphase FISH, on the other hand, can readily detect structural rearrangements in addition to aneuploidy. Furthermore, because metaphase FISH, like classical cytogenetics, analyzes dividing cells, the results from these two methods may be directly compared. A number of studies have determined that FISH is both more sensitive and convenient than classical cytogenetics [Poddighe et al., 1991; Kadam et al., 1993; Kibbelaar et al., 1993]. Therefore, FISH appears to be the more suitable method for large-scale population biomonitoring, which requires a convenient assay with the ability to analyze many cells from a large number of subjects in the general population.

Using metaphase FISH, we have recently found that the peripheral blood cells of workers exposed to benzene have higher levels of leukemia-specific aberrations, such as t(8; 21) [Smith et al., 1998], del(5q), del(7q), and monosomy and trisomy 7 [Zhang et al., 1998a]. As part of this ongoing investigation, we have also used interphase FISH to detect aneusomies in the C-group chromosomes 7, 8, and 9 and have previously published the data for chromosome 9 [Zhang et al., 1996a]. The fact that aneusomies of chromosomes 7 and 8 were determined by both metaphase and interphase FISH allowed us to compare the data produced by these two methods. The aim was to establish which approach was more sensitive in the detection of benzene-induced aneuploidy, a potential biomarker of early effect, at lower levels of exposure. Our data show that metaphase FISH is more sensitive than interphase FISH in detecting both benzene-induced monosomy and trisomy.

MATERIALS AND METHODS

Subject Enrollment

Identification of factories and enrollment of study subjects have been previously described in detail by Rothman et al. [1996]. Biological samples were collected from 44 healthy workers currently exposed to benzene with minimal exposure to toluene and other aromatic solvents in Shanghai, China, in October 1992. The same number of healthy controls without current or previous occupational exposure to benzene were enrolled from factories in the same geographic area. Controls were frequency-matched to the exposed subjects by gender and age (5-year intervals). Exclusion criteria for all subjects were history of cancer, therapeutic radiation, chemotherapy, or current pregnancy. Factories for exposed subjects were selected so that the study population would have a wide range of exposures to benzene similar to exposure patterns in a larger cohort study [Yin et al., 1996; Hayes et al., 1997].

The protocol was explained to all potential participants and informed consent was obtained using Institutional Review Board–approved procedures. Each subject was administered a questionnaire by a trained interviewer. Data collected included age, sex, current and lifelong tobacco use, current alcohol consumption, medical history, and work history. Height and weight of each subject were measured. Peripheral blood was obtained by phlebotomy; blood from one individual could not be successfully cultured and FISH results are reported here for 87 subjects.

Exposure Assessment

Individual exposure was monitored by organic vapor passive dosimetry badges (3M #3500, St. Paul, MN), which were worn by each worker for a full workshift on 5 separate days during the 1 to 2 week period prior to phlebotomy. Badges were analyzed by gas chromatography with flame ionization detection. An 8-hr time-weighted average (TWA) exposure was calculated for benzene as the geometric mean of the five air measurements. Current exposures to benzene were confirmed by the analysis of phenol and other metabolites in urine [Rothman et al., 1996]. A detailed assessment of factory records and operations showed that no other known marrow-toxic chemicals or physical agents were present in these workplaces. Historical benzene exposure during subjects' employment at the study factories was estimated using work histories obtained by interview, company employment records, and factory records as previously described [Rothman et al., 1996]. All exposure assessment was performed blinded with respect to FISH analysis.

Personal benzene air levels in these factories were much higher than had been expected, based on historical area monitoring data. As a direct result of this study, remedial action was taken at the two workplaces with the highest benzene exposures and included substitution of toluene for benzene, enclosure of reaction vessels, and improvement in ventilation.

Blood Cell Cultures and Slide Preparation

Whole blood collected in a vacutainer with the anticoagulant heparin was cultured in RPMI-1640 medium supplemented with 15% fetal bovine serum, 1% penicillin-streptomycin, 1% L-glutamine (Gibco, Grand Island, NY), and 1% phytohemagglutinin-P (Pharmacia, Piscataway, NJ). Blood cells were incubated at 37°C in a 5% CO_2 moist atmosphere and harvested at 72 hr after culture initiation. Colcemid (0.1 μg/ml) was added 4 hr prior to harvest to obtain a sufficient number of metaphase spreads (for metaphase FISH only). After hypotonic treatment (0.075 M KCl) for 30 min at 37°C, the cells were fixed three times with freshly made Carnoy's solution (methanol:glacial acetic acid = 3:1). The fixed cells were then dropped onto prelabeled glass slides, allowed to air-dry, and stored at −20°C under a nitrogen atmosphere. Prepared slides were later shipped on dry ice to the United States.

Fluorescence in Situ Hybridization (FISH)

Metaphase FISH

A digoxigenin-labeled chromosome 7 probe and a biotinylated human painting probe specific for chromosome 8 were purchased from Oncor Inc. (Gaithersburg, MD). The two probes were used separately on metaphase spreads prepared from cultured lymphocytes. The chromosome 8 painting probe was prewarmed for 5 min at 37°C, denatured for 10 min at 70°C, and

then preannealed for 2.5 hr at 37°C prior to hybridization with cellular DNA. The chromosome 7 probe, on the other hand, needed no preannealing prior to hybridization. Denaturation of cellular DNA, hybridization with DNA probes overnight, postwashing of slides, and detection of hybridization signals were the same as recently described in detail [Smith et al., 1998; Zhang et al., 1998b].

Interphase FISH

A digoxigenin-labeled human centromeric cocktail probe specific for chromosome 7 (α-satellite) and a biotinylated centromeric probe for chromosome 8 (α-satellite) were also purchased from Oncor Inc. A dual-color hybridization of chromosomes 7 and 8 was performed in the interphase cells for this study. Detailed procedures for FISH with repetitive DNA probes have been described previously [Zhang et al., 1994, 1996a]. Briefly, the chromosome probes were mixed with sonicated salmon sperm carrier DNA in Master Mix 2.1 solution (55% formamide/1×SSC/10% dextran sulfate). The probe DNA was denatured at 70°C for 5 min and cellular DNA denatured in 70% formamide in 2×SSC (0.3 M sodium chloride and 0.03 M sodium citrate, pH 7.0) at 72°C for 2 min. After rapid removal to ice, the mixture of probes was then hybridized with target DNA overnight at 37°C in a humidified chamber. The slides were then postwashed in 0.5×SSC at 72°C for 5 min followed by three brief washes in 0.1 M phosphate buffer (pH 8.0) at room temperature. The hybridization signals were detected in a dual-color detection solution with 10 μg/ml rhodamine-conjugated antidigoxigenin antibody (Boehringer-Mannheim, Indianapolis, IN) and 10 μg/ml FITC-avidin (Vector, Burlingame, CA) in phosphate buffer for 15 min at 37°C. After the slides were washed three times for 2 min at a time in phosphate buffer with intermittent agitation at room temperature, the nuclei of cells were counterstained with a blue fluorescent dye 4,6-diamino-2-phenylindole (DAPI, 0.1 μg/ml; Sigma, St. Louis, MO) prepared in a mounting medium (Vector).

Aneusomy Detection and Criteria

The hybridization signals were viewed by a Nikon fluorescence microscope equipped with epifluorescent illumination and a 100× oil immersion lens. A triple-bandpass filter for DAPI/FITC/Texas Red (excitation at 405, 490, and 570 nm; emission at 460, 525, and 635 nm) was used. The digoxigenin-labeled probe was stained red (chromosome 7) and the biotin-labeled probe, green (chromosome 8). Normal interphase cells had two red and two green signals on a blue nucleus background. All the stained slides were randomized and coded prior to scoring. Samples of 1000 cells per subject were scored for the presence of fluorescent probe signals in each nucleus. Both the number of interphase nuclei with zero, one, two, three, four, and five or more spots per probe and the total number of scored cells were recorded on a specially designed scoring sheet. Detailed scoring criteria for interphase cells were described previously [Zhang et al., 1994]. In general, over 99.5% of the interphase cells showed one, two, three, or more in situ hybridization signals in all preparations.

For efficiency, all scorable metaphase spreads on each slide were analyzed and a minimum of 200 cells per subject were scored. Metaphase cells were considered scorable if they met specific criteria [Smith et al., 1998; Zhang et al., 1998b]. The total numbers of scored metaphase spreads from the 87 subjects were 30,200 for chromosome 7 (average 347 cells/subject) and 42,082 for chromosome 8 (average 483 cells/subject).

Statistical Analyses

Study subjects were divided into controls, workers exposed to 31 or fewer ppm benzene as an 8-hr TWA (the median exposure), and workers exposed to more than 31 ppm as an 8-hr TWA in order to study dose-response relationships and the ability of each technique to detect differences between the lower exposed and unexposed controls. We have previously shown that these exposure categories strongly correlate with urinary benzene metabolite levels and various measures of hematotoxicity [Rothman et al., 1996]. Initial analyses comparing aneuploidy frequencies in all exposed workers to those in controls led to the same overall conclusions and are not presented in this report. Summary data for aneuploidy of chromosomes 7 and 8 within these exposure groups are presented by the mean of the number of aberrations per 100 cells scored for each subject and the standard error. Each measure was normalized by a square root transformation and a test for trend was performed by linear regression. Aneuploidy values in controls were compared to values in lower- and higher-exposed workers by analysis of variance. All models contained age and sex, the original matching variables. There was no evidence of confounding by current or previous tobacco use, alcohol use, or body mass index, and these were excluded from final models. Spearman rank order correlation was used to test the relationship between the interphase and metaphase FISH assays.

RESULTS

Demographic Characteristics of the Study Populations

Exposed workers (n = 44) and controls (n = 44) had similar demographic characteristics. Forty-eight percent of each group were women. The mean age of the exposed group was 35.3 ± 7.8 (mean ± SD) and that of the controls was 35.4 ± 7.3. None of the women in either group smoked, but 21 of 23 men in both the exposed and control groups were smokers. The exposed males smoked 11.0 ± 7.8 cigarettes/day and nonexposed smoked 13.5 ± 13.7 cigarettes/day. Both groups had a low mean alcohol intake: 1.5 ± 3.2 drinks/week among exposed workers and 1.4 ± 2.2 drinks/week among controls. The workers exposed to benzene had worked in their respective factories for a mean of 6.3 ± 4.4 years, with a range of 0.7 to 16 years.

Comparison of Metaphase and Interphase FISH in Detecting Cells With One Hybridization Signal

In this study we applied both metaphase and interphase FISH assays to detect aneusomy of chromosomes 7 and 8 in the lymphocytes of workers exposed to benzene and matched controls. The frequency (%) of cells with one hybridization signal for chromosome 7 in metaphase spreads rose from 2.72 ± 0.19 (mean ± SE) in controls to 3.79 ± 0.63 (39% increase, not significant [NS]) in workers exposed to 31 or fewer ppm benzene and 5.9 ± 0.85 (117% increase, $P < 0.0001$) in workers exposed to more than 31 ppm benzene ($P_{trend} < 0.0001$, Fig. 1a). In contrast, interphase FISH detected no evidence of a dose-response relationship in the frequency of cells with one hybridization signal for chromosome 7 (controls, 15.95 ± 1.07%; ≤31 ppm, 15.58 ± 1.29; and >31 ppm, 17.22 ± 1.51, Fig. 1a). The frequencies of cells with one hybridization signal for chromosome 7 detected by interphase FISH were substantially higher in all groups than by metaphase FISH (e.g., 15.95 vs. 2.72, respectively, among controls), probably due to probe overlap artifact in interphase cells.

Similarly, the frequency of cells with one hybridization signal for chromosome 8 in metaphase spreads rose from 5.76 ± 0.48 in controls to 6.26 ± 0.59 (8.7% increase, NS)

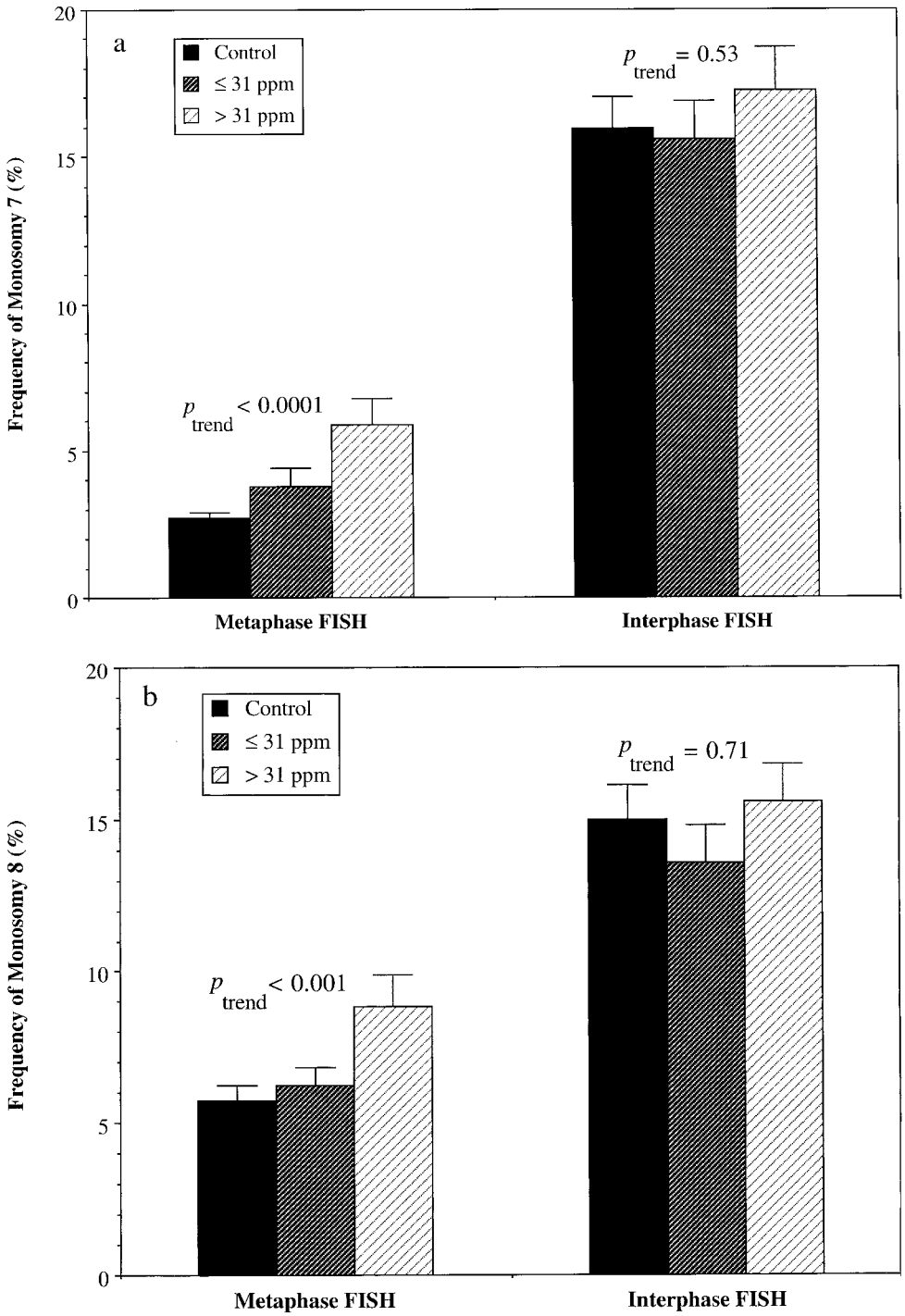

Fig. 1. Monosomy of chromosomes 7 (**a**) and 8 (**b**) detected by both metaphase and interphase FISH in Chinese workers exposed to benzene and matched controls. Data presented are mean monosomy frequency (%). Error bar represents SE. Black bar represents control, dark shaded bar represents 31 or fewer ppm group, and light shaded bar represents greater than 31 ppm group. A significant dose response was observed in monosomy 7 (a) in metaphase cells ($P_{trend} < 0.0001$), but not in interphase cells. Likewise, a significant dose response in monosomy 8 (b) was observed in metaphase cells ($P_{trend} < 0.001$), but not in interphase cells.

in workers exposed to 31 or fewer ppm benzene and 8.85 ± 1.03 (54% increase, $P < 0.001$) in those exposed to more than 31 ppm benzene ($P_{trend} < 0.001$, Fig. 1b). Here again, interphase FISH detected no evidence of a dose-response relationship in the frequency of cells with one hybridization signal for chromosome 8 (Fig. 1b). The frequencies of cells with one hybridization signal for chromosome 8 detected by interphase FISH were much higher in all groups compared with those detected by metaphase FISH (e.g., 14.97 vs. 5.76, respectively, among controls).

Comparison of Metaphase and Interphase FISH in Detecting Cells With Three Hybridization Signals

Increased frequencies of cells with three hybridization signals could be detected in benzene-exposed workers by both metaphase and interphase FISH. The frequency of cells with three hybridization signals for chromosome 7 in metaphase spreads rose from 0.87 ± 0.09 in controls to 1.38 ± 0.20 (59% increase, $P < 0.05$) in workers exposed to 31 or fewer ppm benzene and 1.92 ± 0.34 (121% increase, $P < 0.0001$) in workers exposed to more than 31 ppm benzene ($P_{trend} < 0.0001$, Fig. 2a). Similarly, the frequencies of cells

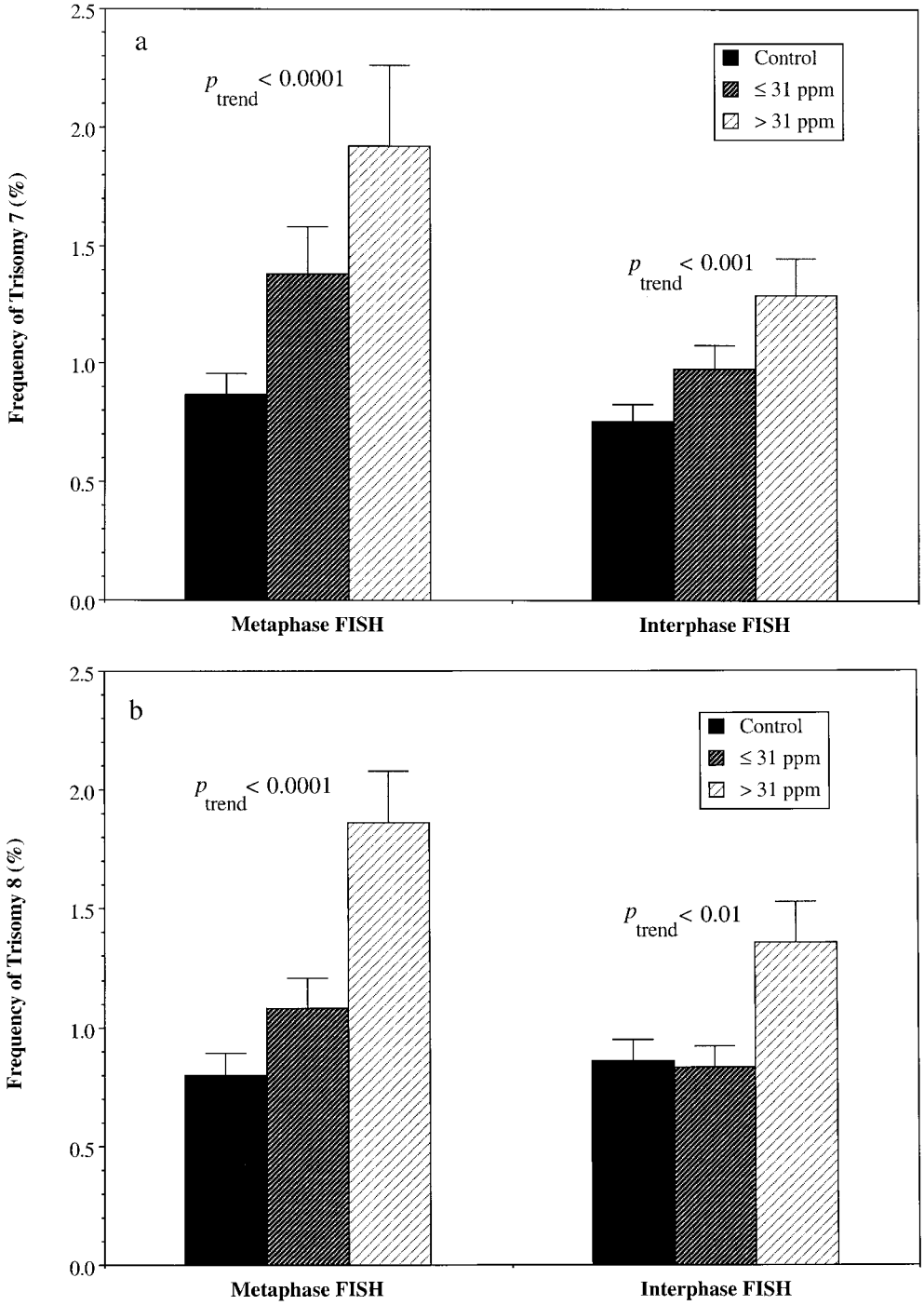

Fig. 2. Trisomy of chromosomes 7 (**a**) and 8 (**b**) detected by both metaphase and interphase FISH in Chinese workers exposed to benzene and matched controls. Data presented are mean trisomy frequency (%). Error bar represents SE. Black bar represents control, dark shaded bar represents 31 or fewer ppm group, and light shaded bar represents greater than 31 ppm group. Significant dose responses were observed in trisomy 7 (a) and trisomy 8 (b) in both metaphase and interphase cells. The significance of the test for trend was higher in metaphase cells for both trisomy 7 and 8 ($P_{trend} < 0.0001$) than in interphase cells ($P_{trend} < 0.001$ for trisomy 7 and $P_{trend} < 0.01$ for trisomy 8).

with three hybridization signals for chromosome 7 detected by interphase FISH showed a significant dose-response relationship ($P_{trend} < 0.001$, Fig. 2a). However, the increase over controls (0.76 ± 0.07) detected in interphase cells of workers exposed to 31 or fewer ppm (0.98 ± 0.10, 29% increase, NS) and more than 31 ppm (1.29 ± 0.16, 70% increase, $P < 0.01$) was somewhat smaller than that detected in metaphase spreads.

Increased frequencies of cells with three hybridization signals for chromosome 8 in benzene-exposed workers could also be detected by both FISH assays. The frequency of cells with three hybridization signals for chromosome 8 in metaphase spreads was increased from 0.80 ± 0.09 in controls to 1.08 ± 0.13 (35% increase, NS) in the 31 or fewer ppm exposure group and 1.86 ± 0.22 (132% increase, $P < 0.0001$) in the greater than 31 ppm group ($P_{trend} < 0.0001$, Fig. 2b). Although the frequency of cells with three hybridization signals for chromosome 8 detected by interphase FISH showed a significant dose-response relationship ($P_{trend} < 0.01$, Fig. 2b), there was essentially no difference between frequencies in the controls (0.86 ± 0.09) and the lower-exposed workers (0.83 ± 0.09), but a significant increase (58%, $P < 0.01$) was detected in the greater than 31 ppm group (1.36 ± 0.17).

Frequency of Cells With Zero and Four or More Hybridization Signals

Frequencies of cells with zero hybridization signals for chromosomes 7 and 8 were very low as detected by both interphase FISH (0.036% and 0.021%, respectively) and metaphase FISH (0.023% and 0.019%). These results indicate that the hybridization efficiency for both chromosomes was very high in both interphase and metaphase cells. Frequencies of cells with four hybridization signals were 0.20 and 0.21%, respectively, for chromosomes 7 and 8 in interphase cells and 0.65 and 0.43%, respectively, for chromosomes 7 and 8 in metaphase spreads. Over 85% of cells with four signals were actually tetraploid by both FISH methods. The frequency of cells with more than four hybridization signals was extremely low: 0.005 and 0.004% by interphase FISH, and 0.026 and 0.024% by metaphase FISH, for chromosomes 7 and 8, respectively.

Correlation Between Interphase and Metaphase FISH

As expected, there was no correlation between interphase and metaphase FISH data for frequencies of cells with one hybridization signal for chromosomes 7 and 8 ($r = -0.01$ and -0.17, NS, respectively). In contrast, there was a modest correlation between interphase and metaphase data for cells with three hybridization signals for chromosomes 7 ($r = 0.28$, $P < 0.01$) and 8 ($r = 0.42$, $P < 0.0001$).

DISCUSSION

FISH is now a widely used tool in the analysis of chromosomal changes in human cancers, including leukemias, and in prenatal diagnostics. It has also been extensively used to analyze chromosomal damage induced by exposure to ionizing radiation. One advantage of FISH over conventional cytogenetics is that it can be utilized to analyze both metaphase spreads and interphase cells. The FISH-based analysis of interphase cells is mostly limited to the detection of numerical chromosome changes or specific rearrangements. Interphase FISH cannot readily detect the more general structural aberrations observable through metaphase analysis. However, it has a number of advantages, including: (1) analysis of nondividing cells; (2) analysis of a larger number of cells; and (3) easy detection of aneuploid cells. Despite these advantages, there have been few direct comparisons of interphase and metaphase data. Therefore, as part of an ongoing biomarker study of benzene-exposed workers in China, we have directly compared the abilities of interphase and metaphase FISH to detect chromosomal changes in the peripheral blood of these otherwise healthy workers. Because exposure to the leukemogen benzene has previously been associated with changes in C-group chromosomes, and aneusomy of chromosomes 7 and 8 are commonly observed in leukemia, we employed FISH to analyze numerical changes in these two chromosomes.

We found that metaphase FISH could sensitively detect loss of chromosomes 7 and 8 associated with benzene exposure, whereas interphase FISH could not. This result is not surprising, given the well-known problem in interphase cells of probe overlap artifact, which makes detection of chromosome loss highly difficult. The overall increase in frequencies of cells with one hybridization signal for chromosome 7 observed by metaphase FISH was greater than that for chromosome 8, and changes at lower levels of exposure were more marked for 7 than for 8. Metaphase FISH also detected significant dose-dependent increases in the frequency of cells with three hybridization signals on chromosomes 7 and 8 associated with benzene exposure. These cells are most likely hyperdiploid and trisomic for at least one chromosome. Again, at lower levels of exposure (≤31 ppm), the increase in the frequency of cells with three hybridization signals for chromosome 7 was greater than that for chromosome 8.

In order to investigate further the sensitivity of metaphase FISH at low-exposure levels, we analyzed the data from a subgroup of seven workers who had an overall 8-hr TWA less than 10 ppm benzene (based on 5 days of measurements, none of which exceeded 30 ppm). The frequency of cells with three hybridization signals on chromosome 7 detected by metaphase FISH in this subgroup (1.7 ± 0.21) was significantly higher (95% increase, $P < 0.01$) than in controls (0.87 ± 0.09). In contrast, there was no evidence that any other measure of aneuploidy differed between this subgroup and controls. These results suggest that chromo-

some 7 is more sensitive to the effects of benzene than chromosome 8, especially at lower-exposure levels. The reasons for this apparently greater sensitivity remain unclear at this time, but we have previously observed that chromosome 7 is more sensitive to the aneuploidy-inducing effects of benzene than chromosomes 1 and 5 [Zhang et al., 1998a]. Potential explanations for this phenomenon may include the possibility that benzene and its metabolites cause greater telomere elimination on particular chromosomes, which has been shown to lead to selective chromosome loss [Sandell and Zakian, 1993]. Our published results showing that benzene and its metabolites increased telomere elimination on chromosome 7 along with the loss of the chromosome in healthy exposed workers and in cultured lymphocytes support this hypothesis [Zhang et al., 1998a,b]. Further, it has been shown that telomerase, an enzyme that synthesizes telomeric DNA onto chromosomal ends, was highly activated among acute myeloid leukemia patients with monosomy 5 and 7 cytogenetics [Zhang et al., 1996b]. Another potential explanation for the apparently greater sensitivity of chromosome 7 is that the spatial positioning of the chromosomes during prometaphase may be disrupted by benzene exposure, leading to selective chromosome gain and loss [Nagele et al., 1995].

Interphase FISH was also able to detect significant dose-dependent increases in frequencies of cells with three hybridization signals for chromosomes 7 and 8, but the changes detected were less marked than those observed by metaphase FISH. The reason for this result may be that interphase FISH examines noncycling as well as cycling cells, whereas metaphase spreads necessarily include only cycling cells. Cycling cells would be expected to have higher levels of aneuploidy than noncycling cells because genetic damage caused by the chemical exposure has been expressed during division. The lower sensitivity of interphase FISH is not explained by a lower hybridization efficiency in interphase cells than in metaphases. The hybridization efficiency in interphase cells was greater than 99.5%. Interphase FISH could not detect changes in the frequency of cells with one hybridization signal, mostly because of the problem of probe overlap, which is responsible for up to 90% of observable monosomy detected by interphase FISH [Eastmond and Pinkel, 1990]. Thus, there was no correlation between frequencies of one hybridization signal detected by metaphase and interphase FISH. There was, however, a modest correlation between the frequencies of cells with three hybridization signals detected by the two methods.

The generally lower sensitivity of interphase FISH in detecting changes in the frequency of cells with three hybridization signals suggests that this technique may need to be modified for monitoring workers exposed to levels of benzene lower than those studied here, which were quite high (median TWA = 31 ppm; U.S. permissible exposure level = 1 ppm). Metaphase FISH, on the other hand, appears more sensitive and may be a useful method for monitoring workers exposed to concentrations of benzene at or near occupational standards in this and other countries. We are currently planning additional biomarker studies of lower-exposed workers to test further the utility of metaphase FISH in the biological monitoring of benzene exposure. Other groups are working on improving the sensitivity of interphase FISH by using bromodeoxyuridine to label cycling cells, allowing for differentiation of cycling and noncycling interphase cells. This appears to be a very promising approach.

One issue that must be addressed is the apparently high rates of monosomy (cells with one hybridization signal) and trisomy (cells with three hybridization signals) reported in metaphase cells in the present study compared with rates obtained by classical cytogenetics. The high rates of apparent monosomy and trisomy most likely result from the fact that we examined all scorable metaphases on the slides, as previously defined [Zhang et al., 1998b], rather than just the 50 best. This approach accounts for the difference between our data and the much lower numbers generated by conventional analysis of a limited number of high-quality metaphase spreads. When we reexamined around 50 of the best metaphase spreads by FISH in three subjects with high aneuploidy rates, we detected very few aneuploid cells. In fact, on average, the rate of aneuploidy was several-fold lower in the best 50 metaphase spreads compared with those of lower quality (data not shown). Therefore, using FISH to analyze only the best quality spreads would have yielded a lower aneusomy rate comparable to rates obtained by classical cytogenetics. The high values we report here and elsewhere result from our scoring poor-and medium- as well as high-quality spreads. This approach appears to increase our power to detect chromosomal damage in exposed populations, but does not allow for ready comparison of our data with measurements of aneuploidy by conventional cytogenetics or even by FISH in the best metaphase spreads.

The high values of approximately 15% for interphase cells with one hybridization signal result from the fact that a rapid scoring approach with nonrestrictive criteria was applied. According to our criteria, where two hybridization signals overlap and cannot be separated by adjusting the focus, they are counted as one signal. This leads to a high level of apparent monosomy, more than 80% of which probably reflects probe overlap artifact and not “true” monosomy. The application of more restrictive criteria and extensive efforts to separate signals can reduce the average “monosomy” levels to 4% or less. Thus, our assessment of the ability of interphase FISH to measure chromosome loss may have underestimated its value relative to metaphase FISH. Efforts should be made to improve the ability of interphase FISH to detect chromosome loss, since it is applicable to many more cell types than in metaphase FISH. However, in dividing blood cells, the high sensitivity of metaphase FISH makes it the method of choice for analysis of chromosome loss.

Finally, this and prior reports from our group [Zhang et

al., 1996a; Smith et al., 1998; Zhang et al., 1998a] clearly demonstrate that benzene exposure is associated with increased levels of aneuploidy at doses that do not cause overt hematotoxicity. Whereas previous studies have demonstrated aneuploidy in workers with benzene poisoning and severe clinical symptoms [Pollini et al., 1969; Forni et al., 1971], several major studies applying conventional cytogenetics in healthy workers exposed to lower benzene levels have been unable to detect chromosome changes [Yardley-Jones et al., 1990; Major et al., 1994; Tompa et al., 1994]. The limited sensitivity of classical cytogenetics probably explains its inability to detect aneuploidy, which we have shown can be detected by both interphase and metaphase FISH, in otherwise healthy workers exposed to benzene. In particular, metaphase FISH holds promise as a biomarker of early effect in detecting aneuploidy in workers exposed to levels even lower than those studied here.

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